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CHARACTERIZATION AND DRUG DELIVERY OF AALTOCELL™ MICROCRYSTALLINE CELLULOSE

Master´s Programme in Chemical, Biochemical and Materials Engineering

Major in Functional Materials

Master's thesis for the degree of Master of Science in Technology submitted for inspection, Espoo, 12.03.2018

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Abstract

Microcrystalline cellulose (MCC) is widely utilized in various fields, such as food, pharmaceutics, medicine, and cosmetics. As such an important component, MCC has been deeply studied during recent decades. In this thesis, studies focused on AaltoCell™ MCC, which has been manufactured by a novel AaltoCell™ method. This method is more environmentally friendly than the traditional manufacturing methods, which makes this MCC worthy to be researched for applications.

The main tasks of this thesis were characterizing rheological properties of different grades of AaltoCell™ MCC and applying them in controlled drug delivery system as the matrix material. As comparison, a commercial grade of MCC, Avicel® PH-101 was studied. Three types of rheological experiments were conducted to AaltoCell™ MCC, oscillatory stress sweep, frequency sweep, and dynamic viscosity measurement. In the drug release experiments, metronidazole and lysozyme were used as model compounds whose release rates form the gel-like AaltoCell™ matrices were studied.

The results of the rheological experiments indicate that rheological properties strongly depend on the concentration of AaltoCell™ MCC, which means that with increasing the concentration, the rheological properties are significantly increased. The results of the drug release experiments indicate that AaltoCell™ MCC could efficiently control diffusion of both large and small molecule which shows great potential for a drug delivery application.

In further study, the release profiles of other compounds and effect of concentration on the release profiles could be studied.

Keywords Microcrystalline cellulose; Rheology; Drug release; Diffusion coefficient; Hydrogel

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Espoo, March 2018

Yujiao Dong

Content

1. Introduction

Microcrystalline cellulose (MCC) is widely utilized in various fileds, for instance, food, pharmaceutical, medical and cosmetic. Thus, millions of tons MCC has been produced in chemical pulp mills each year. In this thesis work, AaltoCell™ microcrystalline cellulose was studied, which was manufactured by AaltoCell™ method. AaltoCell™ method is a novel manufacturing method, which has been reported (Vanhatalo 2017) more environmentally friendly than traditional manufacturing methods when considering global warming potential as the impact class. The study about the AaltoCell™ method also indicated that MCC can be manufactured under conditions, which can be achieved in industrial scale process. In addition, a new MCC product, which contains lignin, was developed by this novel manufacture method. Thus, the applications of final products have great potential.

The main tasks of this thesis were characterizing the different grades of MCC manufactured by AaltoCell™ method and applying them in pharmaceutical field. In pharmaceutical applications, the dosage forms need specific flow properties in order to be manufactured. Thus, the rheology is the critical property since it will directly influence not only the formulation and efficacy of drug, but also the quality and cost of the final products (Mastropietro, Nimroozi et al. 2013). Therefore, three types of rheological experiments were conducted to the AaltoCell™ MCC, oscillatory stress sweep, frequency sweep and viscosity test. Furthermore, the pharmaceutical application was focused on the controlled drug delivery system. The drug release experiments were carried out to study the release profiles of metronidazole and lysozyme as model compounds.

2. Literature Review

Microcrystalline cellulose used in this thesis is a well-known pure cellulose, which has been utilized in various industries. Furthermore, cellulose as an abundant material with a huge cellulosic material family has been studied for years. The characterization and utilization of cellulosic materials have been reported in various different ways. The application studied in this work, drug delivery system is also significantly relevant to daily life. Hence, drug delivery system has been researched and developed during past several decades. This chapter is a review of earlier studies of cellulosic materials and drug delivery systems, including the rheological properties characterization and the applications.

2.1. Cellulosic Materials

2.1.1. Cellulose and its Derivatives

Cellulose as an abundant organic compound is a natural long chain polymer, which has versatile uses, as well as its derivatives, in many industries, for instance, the food, fibers, cosmetic and especially, pharmaceutical industries (Marques-Marinho, Vianna-Soares 2013, Chang, Zhang 2011, Gibis, Schuh et al. 2015). Various forms of cellulose are different from the shape, size and degree of crystallinity. Microcrystalline cellulose is a well-known pure cellulose with varying grades. The physical and chemical properties of varying MCC grades are strongly depended on the particle size and degree of crystallization, as well as the environmental conditions, for instance, pH, temperature, *etc.* (Nakai, Fukuoka et al. 1977, Trache, Hazwan Hussin et al. 2016, Mathew, Oksman et al. 2005, Baturenko, Chernoberezhskii et al. 2003). Various new grades MCC are manufactured with improved properties by colloidal silicon dioxide or special chemical treatment, such as silisified MCC and second-generation MCC grades (Shokri, Adibkia 2013).

Cellulose ether derivatives are produced by replacing the hydrogen atoms with alkyl or substituted alkyl groups. The molecular weights, chemical structure and distribution of the substituent groups determine the properties of cellulose ethers, for instance, the solubility, viscosity and stability (Shokri, Adibkia 2013). The mostly used cellulose ethers are methyl cellulose (MC), carboxymethyl cellulose (CMC), and sodium carboxymethly cellulose (NaCMC).

Cellulose ester derivatives are classified as organic and inorganic groups. Organic cellulose esters are more important in the commercial applications compared with inorganic cellulose esters (Shokri, Adibkia 2013). Cellulose acetate (CA), cellulose acetate phthalate (CAP) and cellulose acetate butyrate (CAB) are examples of mostly used cellulose esters in commercial applications.

With the increasingly demanding products, as well as the development of techniques, the natural cellulose-based materials came into a new generation with nanoscale. Cellulose nanoparticles are ideal materials for the biopolymer composites industry (Moon, Martini et al. 2011, Eichhorn, Dufresne et al. 2010). Nanofibrillated cellulose (NFC) is one of the well-known nanocellulose materials. Bacterial cellulose is also the product of the development of the biotechnology and biopolymer (Abeer, Mohd Amin et al. 2014). Furthermore, over the past several decades, extensive researches have been reported in cellulose-based particles, cellulose-based composites and cellulose-based hydrogels (Chang, Zhang 2011, Marci, Mele et al. 2006).

2.1.2. Rheological Properties of Cellulosic Materials

The rheology is defined as the study of the flow and deformation of materials under controlled testing conditions with particular emphasis on flow, and the flow is resisted by viscosity (Barnes 2000). In the pharmaceutical manufacturing, the medication products always require specific flow properties to be manufactured. It can be concluded (Mastropietro, Nimroozi et al. 2013, Kutschmann 1998b) that during every stage of manufacturing, the flow property of materials are effected by various factors. Not only in the pharmaceutical, but also in other fields, for instance, in the food industry (Mihranyan, Edsman et al. 2007, Kutschmann 1997), rheology is studied to match the different requirements. As the result, extensive studies about the rheology of materials have been carried out during recent decades.

As mentioned above, there are many factors that can influence the rheology, hence, various studies of varying conditions have been carried out. Rheology study of cellulose nanofibrils (CNFs) suspensions was reported that exhibit the shear thinning and thixotropic behavior (Nechyporchuk, Belgacem et al. 2016). Furthermore, the strength of these networks increases with the increasing cellulose

concentration. Over the varying studies, the emphasis is not only focused on the various conditions of material itself, for instance, the concentrations (EDALI, ESMAIL et al. 2001), but also focused on the different method for characterization (Kutschmann 1998a). The rheology of MCC were reported as effect of addition water and different raw materials (Staniforth, Baichwal et al. 1988, Loo, Mohd et al. 2016).

2.1.3. Applications of Cellulosic Materials

As well known, the cellulosic materials have been directly and indirectly employed in human life cycle as an important role for years. The versatile applications of cellulosic materials determined by the varying properties of materials. For instance, the combination of high strength, stiffness and low weight, biodegradability, renewability, obtains increasing attention to the nano-scale cellulose production and application in composite materials (Siró, Plackett 2010). The bacterial cellulose is an investigated subject in biomedical applications, reinforcement in nanocomposites, electronic paper and fuel cell membranes and the microfibrillated cellulose (MFC) aerogels stimulate the interest in the catalysis, filtration and functional packaging (Lavoine, Desloges et al. 2012). The cellulosic materials can be chemically modified in homogeneous or heterogeneous conditions to manufacture “smart” materials for different applications, which can response to environmental stimuli such as temperature, pH, electricity, *etc.*(Qiu, Hu 2013, Harsh, Gehrke 1991). MCC as a well-known cellulose is an important composition as a stabilizer, fat substitute, binder and emulsifier in food, beverage, pharmaceutical and cosmetic industries (Trache, Hazwan Hussin et al. 2016).

Cellulose and its derivatives are extensively utilized in pharmaceutical industries for different applications (Shokri, Adibkia 2013, Marques-Marinho, Vianna-Soares 2013, Yan, Zhang et al. 2016). Especially the cellulose ethers are widly used in bioadhesives and mucoadhesive drug delivery systems, whereas the cellulose acetate (CA), one of cellulose esters, is the mostly used polymer in formulations of osmotic drug delivery systems(Shokri, Adibkia 2013). Furthermore, the cellulose acetate phthalate (CAP), cellulose acetate trimelitate (CAT), hydroxypropylmethyl cellulose phthalate (HPMCP), and carboxymethylethyl cellulose (CMEC) are commonly used in enteric coated solid dosage forms due to the pH-dependent

property (Shokri, Adibkia 2013, Williams, Liu 2000). Although all of cellulose based polymers exhibit good compatibility, however, special grades of MCC with excellent compatibility are applied on the compressibility enhancers. In addition, various types of cellulose and its derivatives, including hydroxylpropylmethyl cellulose (HPMC), NaCMC, CMC, and hydroxyethyl cellulose (HEC) with different molecular weights, are used in formulations of polymeric matrices, which as drug delivery systems are significantly important in developing of modified release dosage forms. Moreover, depended on varying properties, cellulosic materials are applied as stabilizing agents, gelling agents, fillers for solid dosage forms, binders, *etc.*

2.2. Controlled Drug Delivery System

2.2.1. Theory of Controlled Drug Delivery

Drug delivery system is defined as a system for administering the pharmaceutical compound at target site to achieve a therapeutic effect. Target of controlled drug release systems is to control the plasma concentration of the drug after administration. Compared with other modes of release, for instance one dose and repeated doses, the difference between controlled release is shown in Figure 1 (Bajpai, Shukla et al. 2008), the controlled release is steadier during the desired action time.

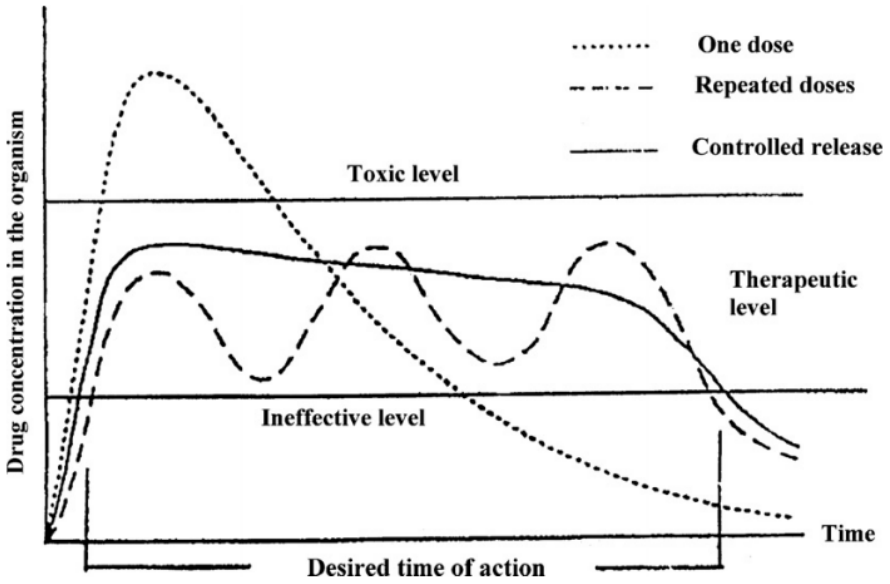

Figure 1. The comparison of controlled release with one dose and repeated doses in plasma concentration (Bajpai, Shukla et al. 2008).

It was reported that the first controlled release formulation was introduced by Keller and Ellenbogen (KELLER, ELLENBOGEN 1952) in 1952 for delivery of d-amphetamine. And after that, controlled drug release technology has been developed for three generations (Park 2014). The first generation is called basics of controlled release, focusing on the basics problems of drug release, for instance the mechanisms of drug release. And decades, the second generation started and focused on the smart delivery systems and smart materials. And now, the third generation is focusing on the modulated delivery systems, which include the targeted delivery and long-term delivery systems.

The controlled drug delivery systems can be categorized by different ways, for instance, the delivery routes, drug types, and release mechanisms. The Figure 2 (Park 2014) shows the classification of drug delivery systems and the development from basic research to clinical applications. According to the Figure 2, although, the started points of each study are different, however, the ultimate goal of drug delivery research is to be applied in the clinical applications.

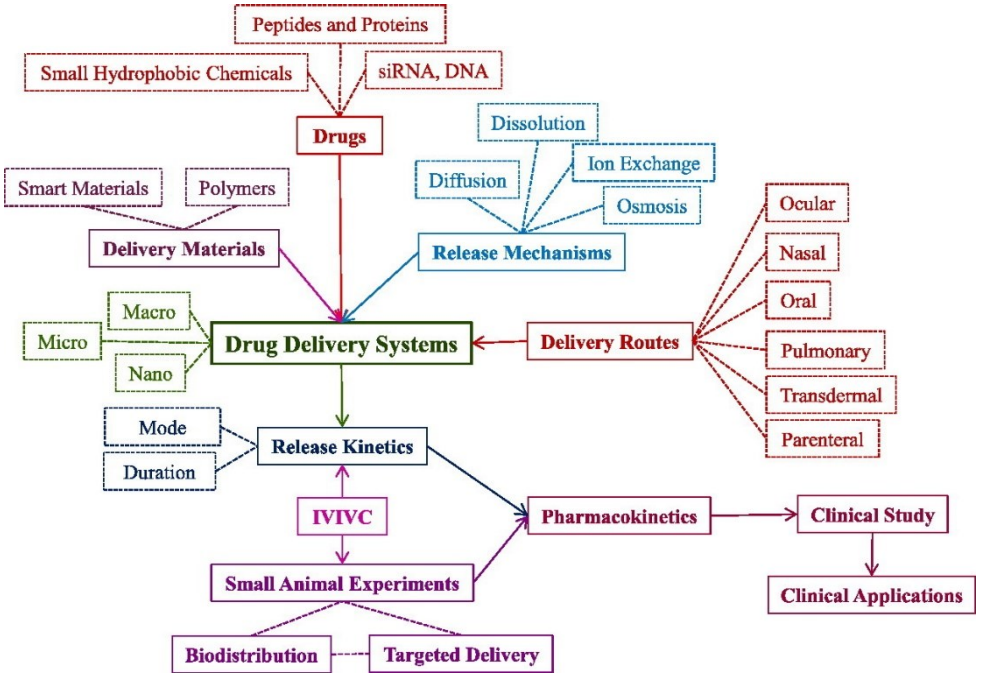

Figure 2. Classification of controlled drug delivery systems and development from basic research to clinical applications (Park 2014).

According to the mechanism controlling the release, drug delivery systems can be divided in four types (Bajpai, Shukla et al. 2008): diffusion-controlled systems; chemically controlled systems; solvent-activated systems; modulated-release systems. The basic release mechanisms are depicted in Figure 3: diffusion controlled, swelling controlled, and chemically controlled. As shown in Figure 3, each mechanism ideally works separately, however, in the real applications, most of delivery devices exhibit the multiple mechanisms (Kiil, Dam-Johansen 2003). In the diffusion-controlled systems, delivery systems can be further divided into different systems according to the inner structure, initial drug content and the geometry which is shown in Figure 4 (Siepmann, Siepmann 2012). In the reservoir systems, the drug is physically separated from the material which controls the release rate, whereas the release rate controlling material and drug are homogeneously distributed in the device within the monolithic systems, which is also known as matrix systems. The matrix systems can be further divided into

monolithic solutions and monolithic dispersions, which decided by the magnitude of initial concentration and solubility.

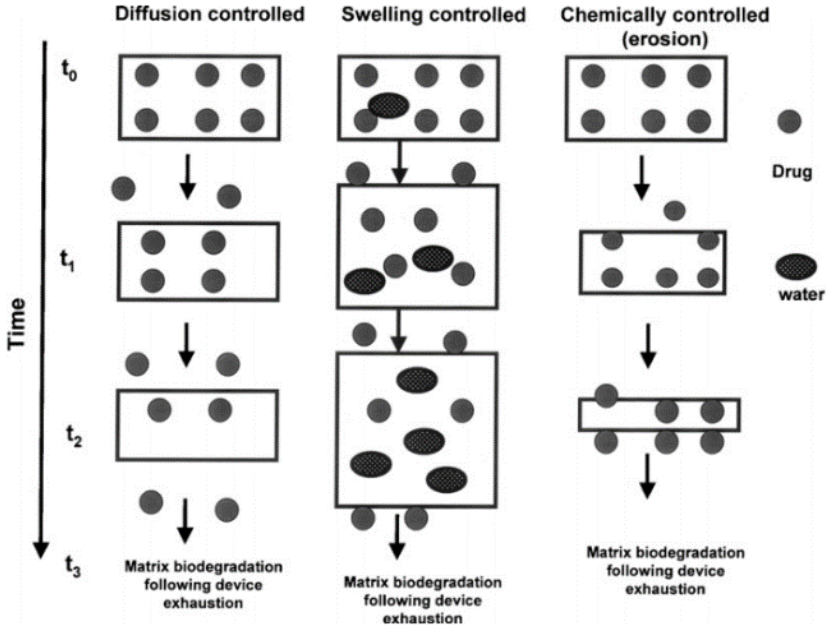

Figure 3. Illustration of three mechanisms for controlled drug release from polymer matrix (Kiil, Dam-Johansen 2003).

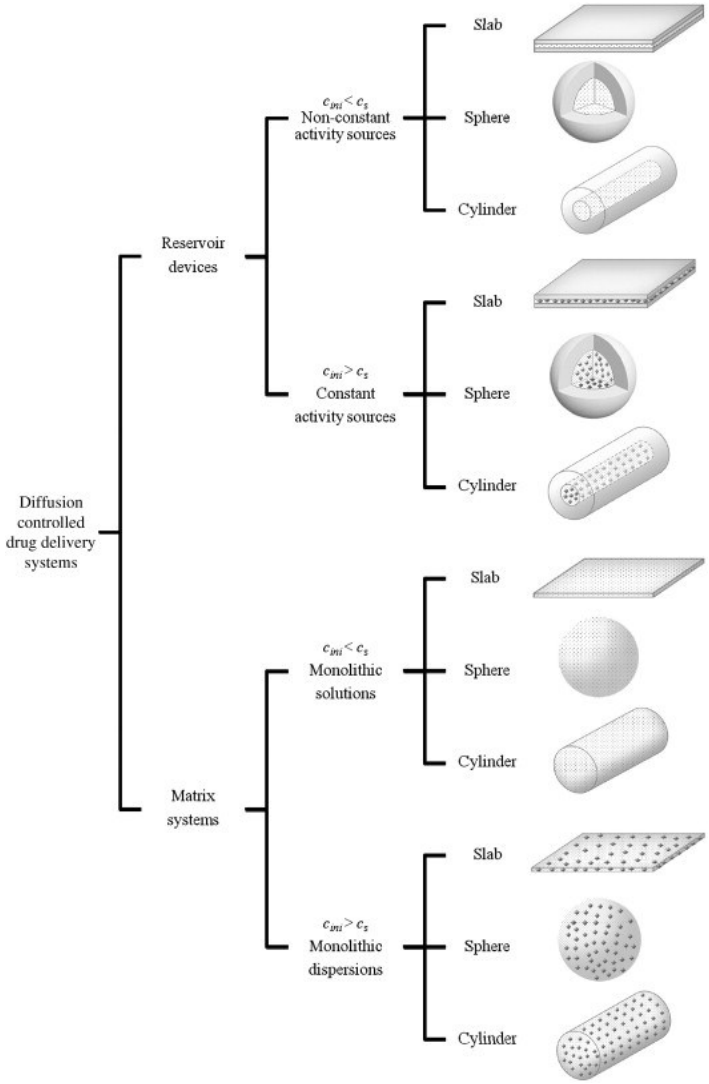

Figure 4. The classification of diffusion controlled drug delivery systems (Siepmann, Siepmann 2012)

In different controlled systems, the methods used to quantify the delivery systems are various. For instance, in swelling controlled systems, the swelling property of matrix material is the significant profile to describe the system. As a diffusion controlled delivery system, the diffusion coefficient of the model compound is used to quantitatively describe the controlled release. In addition, according to the different systems (geometry, inner structure, initial drug content), the principles of calculating diffusion coefficients are different. For instance, in the monolithic dispersion, the initial concentration of drug is much greater than the solubility of the drug, when the geometry is slab, the Higuchi Equation 1 can be used to quantify drug release, which was published in 1961 and became probably the most famous and most often used mathematical equation to describe the release rate of drugs

from matrix systems (Siepmann, Peppas 2001, Higuchi 1961, Siepmann, Peppas 2011):

Equation 1: $M_t = A\sqrt{Dc_s(2c_{ini} - c_s) \cdot t}$

where M_t refers the cumulative absolute amount of drug release at time t; A is the total surface area of the film exposed to the release medium; D is the diffusion coefficient of the drug from the system; c_s is the drug solubility in the wetted matrix and c_{ini} refers to the initial drug concentration in the system.

In the monolithic solution, the initial concentration of drug is less than the solubility of drug, thus, the Fick's second law of diffusion, can be used to solve the different geometries like slabs, spheres and cylinders (Siepmann, Siepmann 2012). For instance, in the slab systems, the cumulative amount of drug released can be calculated by the approximations derived from the infinite series of exponential functions. The approximations are divided into early time and late time, when the $0 < M_t/M_\infty < 0.6$ is called early time and calculated by the Equation 2:

Equation 2: $\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{\pi L^2}\right)^{\frac{1}{2}}$

whereas the $M_t/M_\infty \geq 0.4$ is called late time and calculated by the Equation 3:

Equation 3: $\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} exp\left(-\frac{\pi^2 Dt}{L^2}\right)$

where M_t and M_∞ represent the cumulative amounts of drug released at time point t and infinite time ∞; D is the diffusion coefficient of the drug; L represents the thickness of the hydrogel.

2.2.2. Cellulosic Materials in Drug Delivery System

As mentioned above, different materials with varying properties can be applied to various systems according to the different requirements of systems. Water-soluble diethylaminoethylcellulose was studied in the application of oral drug delivery as an erosion controlled matrix, which is a special type of erosion controlled, called as "polymer particle erosion" (Liesiene, Matulioniene 2004). The research indicated that water-soluble diethylaminoethylcellulose as 6.5 % amount of tablet weight contributed to high mechanical strength and with prolonged drug release. Salama

et al. (Salama, El-Sakhawy et al. 2016) studied carboxymethyl cellulose-based hybrid material indicating the lower swelling percentage compared with pure hydrogel in different pH conditions which possesses the capability to sustained drug release. And microcrystalline cellulose (MCC), hydroxypropylmethyl cellulose (HPMC) and ethyl cellulose (EC) were reported can be produced as both granules and tablets with theophylline as drug model (Chambin, Champion et al. 2004). Hydroxypropyl cellulose (HPC) combined with MCC was reported as a functional polymer used in mucosal drug delivery for peptide delivery (Suzuki, Makino 1999). And many materials can be successfully designed for different proposes by physical or chemical method, for instance, cross-linking. For example, the CMC and cellulose in the NaOH/urea aqueous system can be used to prepare superabsorbent hydrogels by using epichlorohydrin as cross-liner (Chang, Duan et al. 2010). This kind of hydrogels show smart swelling and shrinking in NaCl or CaCl2 solution, moreover, the release profile of bovine serum albumin (BSA) can be controlled by changing the content of CMC. The cationic cellulose hydrogels cross-linked by HEC with ethylenglycol diglycidylether (EGDE) has been reported that exhibited response to pH and ionic strength changing (Rodriguez, Alvarez-Lorenzo et al. 2003).

2.3. Summary of Literature Review

The literature review provides background information about cellulosic materials and characterization methods of the materials, as well as the theory of controlled drug delivery systems. In conclusion, cellulosic materials as abundant materials are significantly important in controlled drug delivery applications which has been deeply studied for over 60 years, however, with the revolution of cellulose materials and the techniques of manufacture, the future of research still has a long way to discover.

3. Material and Methods

This chapter describes materials and methods, which were used for rheological tests and drug release study. The details of materials and the operation conditions and parameters of each experiment in this thesis work are discussed.

3.1. Materials

The AaltoCell™ microcrystalline cellulose used in this thesis was manufactured with AaltoCell™ method described in an earlier publication by Vanhatalo *et al.* (Vanhatalo, Dahl 2014). Three grades of AaltoCell™ microcrystalline cellulose, marked as grade A, B and C and shown in Figure 5, were tested to compare the properties. The difference between three grades are listed in Table 1, grade A was bleached and mechanically dispersed MCC, whereas grade B was bleached but has never been processed through dispersionizer and grade C was unbleached but well dispersed MCC. The dispersionizer processing was carried out by Netzsch Omega® 60 Economic Dispersionizer equipment with 700 bars for processing pressure and under 80 ˚C for temperature. The MCC-water mixture was passed three times through the dispersionizer to form gel material. Commercial microcrystalline cellulose, Avicel® PH-101, was used as a reference material to compare with the AaltoCell™ microcrystalline cellulose.

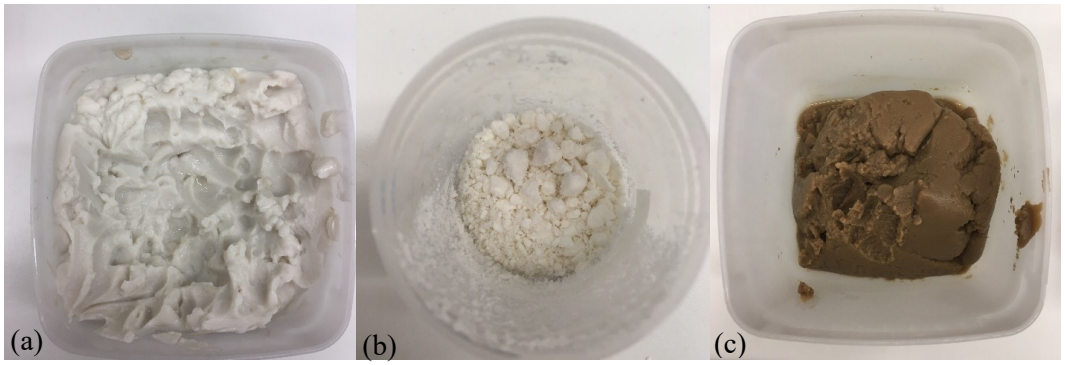

Figure 5. Three different grades of AaltoCell™ microcrystalline cellulose. a, grade A AaltoCell™ MCC with 12% dry content; b, grade B AaltoCell™ MCC with 43% dry content; c, grade C AaltoCell™ MCC with 14% dry content.

Table 1.Comparison of three different grades of AaltoCell™ MCC.

Grade	Dispersion	Bleaching
A	+	+
B	-	+
C	+	-

Metronidazole (MZ) as analytical standard was purchased from Sigma-Aldrich, China. Lysozyme (LZ) from hen egg white was purchased from Roche, Germany. Metronidazole and lysozyme were used as model compounds in this thesis work, representing small molecules and large molecules (M_w ≥1 kDa) separately.

3.2. Methods

3.2.1. Preparation of Rheological Test Samples

All grades AaltoCell™ MCC were diluted to 2.4 %, 5 %, 10 % and 12 % (dry content), as well as the Avicel® was prepared as 10 % (dry content) to test the rheological properties. The dilution was performed by using water and dispersed by the sonicator (Qsonica). The samples of B grade MCC were homogenized in syringes, until the visible blocks cannot be observed. This method was used in the mixture of formulations as well, which is described in next section.

3.2.2. Preparation of MCC Hydrogel Formulations

The preparation of MCC hydrogel formulations were performed in 10 ml syringes. The content of MCC and drug compounds (metronidazole and lysozyme) is presented in Table 2. The drug compounds were added into hydrogels as dry powders. The mixture of hydrogel formulations was prepared by mixing in two attached 10 ml syringes (Figure 6) for 10 min. The MCC amount in the final formulations was 12 % whereas the drug compound concentration was 1 %.

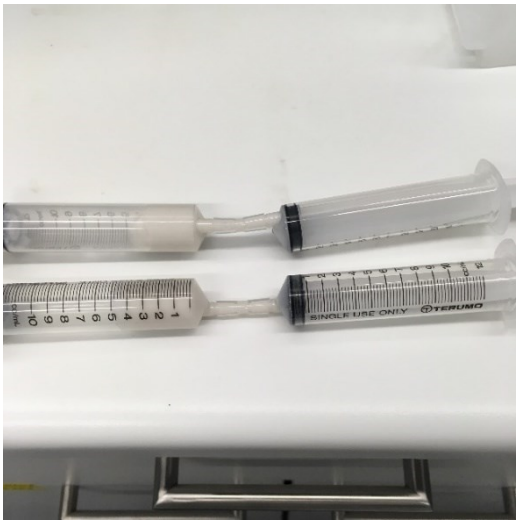

Figure 6. Hydrogel formulations prepared in syringes.

Table 2. The content of MCC and drug compound in formulations prepared for drug release study.

Total mass (mg)	Mass of MCC (mg)	Mass of model compound (mg)	MCC dry content (mg)	MCC content (%)	Model compound (%)	Drug compound with pure MCC (%)
5050	5000	50	600	12	1	8

3.2.3. Rheological Measurements

The rheological measurements were performed at 22 ˚C. The parallel 20 mm diameter steel plate geometry was used with 1000 µm gap with the AR-G2 Magnetic Bearing Rheometer (TA Instruments) equipped with Julabo AWC100 Compact Recirculating Cooler, shown in Figure 7, and results were analyzed with TA data analysis software. Three types of studies were conducted: oscillatory stress sweep, frequency sweep and viscosity test.

Figure 7. The setup of rheometer and cooler system.

The oscillatory stress sweep was conducted over the range of 0.01 Pa to 100 Pa in log mode, at the controlled frequency of 1Hz. For the frequency sweep, samples were subjected to a frequency ranging from 0.01 Hz to 10 Hz at a controlled variable of 5 % strain. The viscosity property was characterized with shear rate range of 0.01

– 1000 s^{-1} for a period of 5 min. All measurements were performed in triplicate and the results were recorded.

3.2.4. Drug Release Experiments

The drug release studies were performed with discs filled with 1.11 g of hydrogel formulations with a constant flat surface area of 1.33 cm^2 exposed to the release buffer. The disc molds were placed in 150 ml amber glass bottles. The bottles were filled in to 70 ml pH 7.4 phosphate buffered saline and kept at 37 ˚C with magnetic stirrer. 10 samples were collected during the 144 h experiment, at 0.5 h, 1 h, 2 h, 4 h, 6 h, 24 h, 30 h, 48 h, 72 h and 144 h. Each time 1.5 ml sample was collected from the bottle and replaced by 1.5 ml fresh buffer.

The equations used to calculate the diffusion coefficients of controlled drug release were different under the different situations, for instance, the ratio of initial drug concentration to drug solubility and the device geometry (Siepmann, Siepmann 2012). In this thesis work, the AaltoCell™ MCC was applied in the drug delivery system controlled by diffusion. Thus, the diffusion coefficient is an important evaluation of the material. The metronidazole has a solubility of 10.5 mg/ml (Wu, Fassihi 2005, Kim, Lee et al. 2012) in the water, and the solubility of lysozyme exceeds 10 mg/ml in water (Fritz, Radmacher et al. 1995, Szymańska, Ślósarek 2012). However, the concentration of both drugs in this study were 1 % which corresponds to 10 mg/ml. Thus, the Fick's second law of diffusion, Equation 2, with early time, when $0 < M_t/M_\infty < 0.6$, could be used for calculations of diffusion coefficients (Siepmann, Siepmann 2012).

3.2.5. Quantification of Model Compounds from Drug Release Samples

The quantification of metronidazole and lysozyme was performed by absorbance measurement with Cary 50 UV-Visible Spectrophotometer (Varian Inc.). For metronidazole, absorbance was measured in 1 cm cell at 320 nm. The calibration curve was from 3 µg/ml to 35 µg/ml. For lysozyme, absorbance was measured in 1 cm cell at 280 nm. The calibration curve was from 2.5 µg/ml to 30 µg/ml. In addition, the squared correlation coefficients were above 0.99 for both calibrations.

4. Results and Discussions

4.1. Samples for Rheological Measurements

For the rheological measurements, the MCC samples were diluted to 2.4 %, 5 %, 10 % and 12 % dry content, and the Avicel® PH-101 sample to 10 %, which is shown in Figure 8. From Figure 8 it can be observed that compared with other grades of MCC, B grade MCC was more liquid-like at 10 % and 12 % concentrations whereas the A grade and C grade were observed as hydrogel. This may due to the A and C grades MCC have been passed dispersion process three times to form gel, whereas B grade MCC was simply dispersed with sonicator. At the same concentration of 10 %, the Avicel® sample can be observed as the suspension, and even layered after a couple of days, on the contrary, the AaltoCell™ MCC was stable at each concentration.

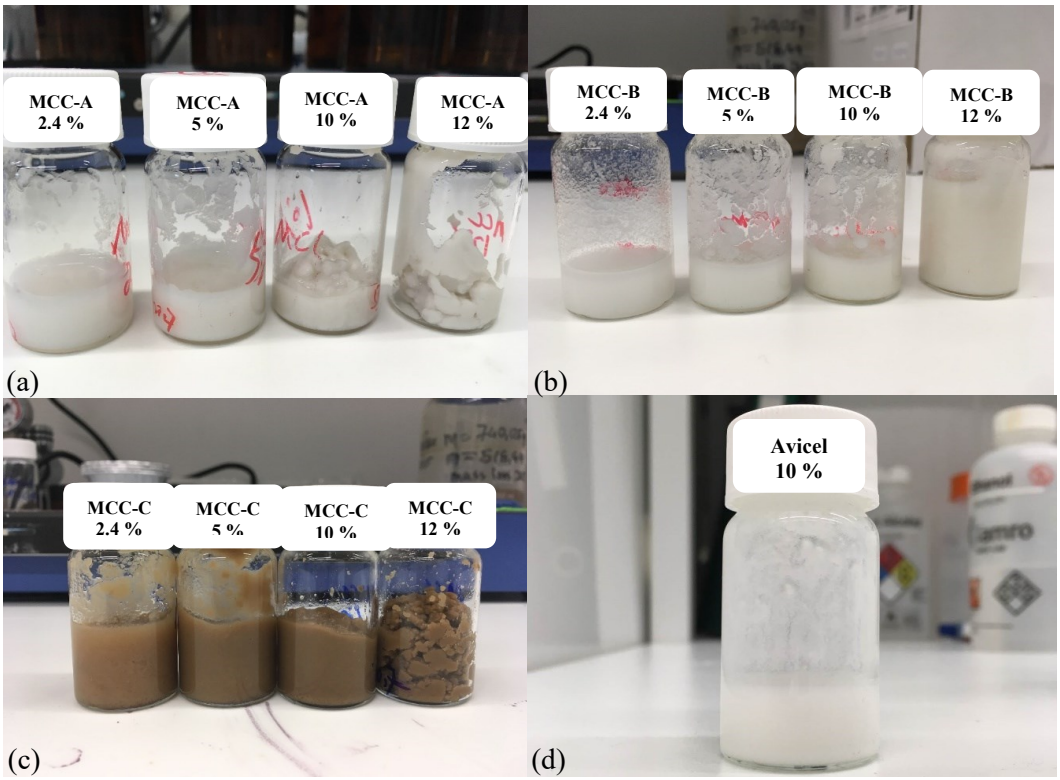

Figure 8. Varying concentrations of diluted MCC samples: a, b, c: 2.4 %, 5 %, 10 %, and 12 % dry content of Grade A,B,C AaltoCell™ MCC; d: 10 % dry content of Avicel® PH-101.

4.2. Oscillatory Stress Sweep

The varying grades and concentrations of MCC were subjected to oscillatory stress sweep. The results are presented in Figure 9. The stress range of the region, where the elastic modulus (*G'*) is independent of the stress, is the linear viscoelastic region and the end of linear region is called the critical stress. At stress below the critical stress, the behavior of the samples is viscoelastic, whereas at the stress over the critical stress, the materials start to yield (Rudraraju, Wyandt 2005b).

The Figure 9a shows the storage modulus of different MCC grades during an oscillatory stress sweep. According to the figure, the C grade exhibits significantly high *G'* values compared to other grades. Furthermore, the grade A and grade C both exhibit longer linear viscoelastic region than B grade and Avicel® have. The Avicel® was yielded below 5 Pa stress and the elastic modulus was less than 10 Pa, whereas the B grade MCC yielded at around 10 Pa stress with over 1000 Pa *G'*. However, A and C grade MCC were still in the linear viscoelastic region at 100 Pa. The error bar of B grade curve is significantly bigger than others are; this phenomenon is caused by the inhomogeneous dispersion during the dilution. The dispersion process also decreased the particle size of MCC, which could enhance the interaction within particles. This interaction enhanced the strength of hydrogel under high stress, which result in higher yield points for grade A and grade C.

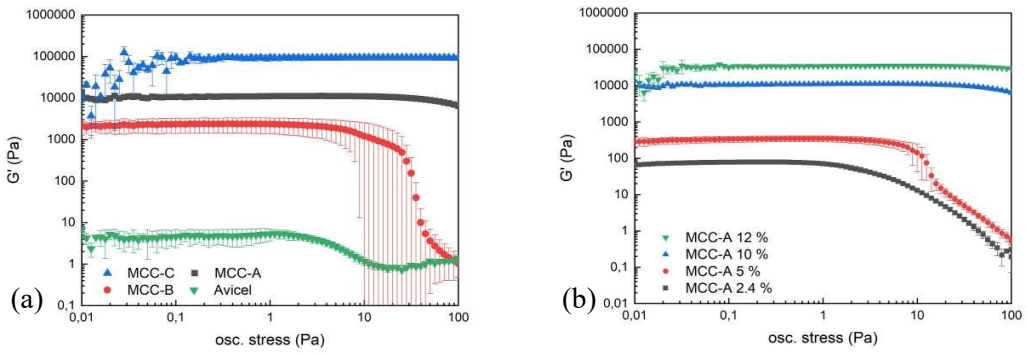

Figure 9. Effect of oscillation stress on the elastic modulus (*G'*) of MCC hydrogels. a, at varying grades; b, at varying concentrations.

Figure 9b shows the effect of different concentrations on the oscillatory stress sweep. All grades of AaltoCell™ MCC were characterized with varying concentrations (2.4 %, 5 %, 10 % and 12 %). The result shown in figure is A grade AaltoCell™ MCC, others are shown in Appendices. According to the Figure 9b,

the results show strong dependency on concentrations and the results of other grades exhibit a similar pattern. The sample having 2.4 % dry weight has the shortest linear viscoelastic region with critical stress around 1 Pa and lowest *G'* values below 100 Pa. With the increasing concentration, the range of linear viscoelastic region and value of critical stress correspondingly increase. At 10 % and 12 % concentrations, the material is still in the linear viscoelastic region at the range of 0.01 Pa to 100 Pa stress. Rudraraju *et al.* (Rudraraju, Wyandt 2005b) studied the rheological properties of microcrystalline cellulose/sodiumcarboxymethyl cellulose (MCC/NaCMC) hydrogels with two commercial grades, Avicel® RC-591 and CL-611. The values of elastic modulus (*G'*) at linear viscoelastic region for 2 % (w/w) MCC/NaCMC hydrogels were below 22.8 Pa, which is quite similar with the result of 2.4 % A grade MCC. However, the critical stress for MCC/NaCMC was 29 Pa, which is higher than 2.4% A grade MCC. This may due to the interaction within the structure of MCC and NaCMC. Also the set up of measurement greatly affects the rheological results.

4.3. Frequency Sweep

The frequency ramp was applied to reveal the storage modulus, *G'* and loss modulus, *G"*, responses of different grades of MCC and varying concentrations as a function of the oscillation frequency as plotted in Figure 10. The loss tangent (*tan δ*) is a dimensionless parameter, which is the ratio of energy lost to the energy stored during the deformation (Rudraraju, Wyandt 2005b). The values of *tan δ* indicate the behavior of materials, when the value is greater than a unit, material is viscous dominant behavior, whereas material is elastic dominant behavior when the value is less than a unit.

The Figure 10a presents the variation of *G'* and *G"* with frequency for different grades MCC. The C grade MCC exhibits the highest value of *G'* and *G"*, followed by A grade MCC and B grade MCC, whereas the Avicel® had the lowest values. The curves of *G'* against the frequency for grade A and C MCC show insignificantly increase with increasing frequency. However, for the Avicel® and B grade MCC, the values of *G'* and *G"* at high frequency are decade higher than the values at low frequency. In addition, at the same concentration, the values of *G'* for Avicel® and

C grade MCC were always higher than the values of *G"*, however, for the A and B grades were opposite that values of *G"* were higher than the values of *G'*. It also can be concluded from Figure 10b; the *tan δ* of grade A and B are higher than a unit and for C grade and Avicel® are less than a unit. This difference between grade A, B with grade C may due to the participation of lignin. Additionally, result of Avicel® shows considerable increase with increasing frequency indicating the material could be dominant viscous, which is proved in the result of *tan δ* as well.

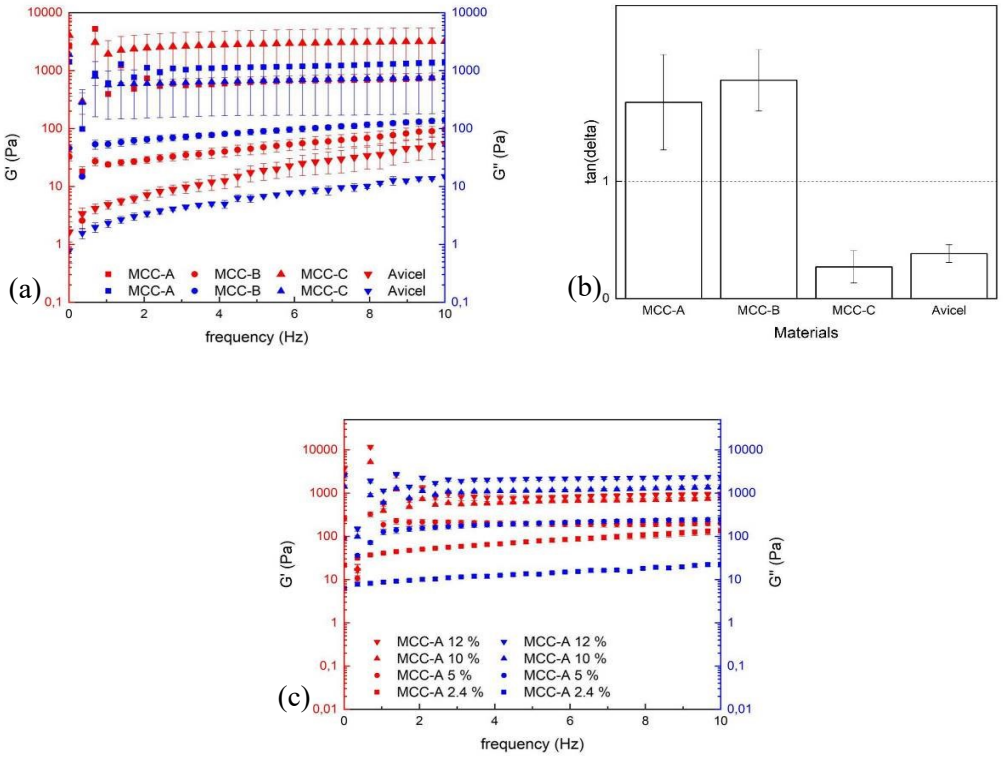

Figure 10.Influence of frequency on the storage modulus (*G'*) and loss modulus (*G"*) of MCC 12 % hydrogels, a, b, at varying grades and *tan δ* of varying grades; c, at varying concentrations.

According to the Figure 10c, the curves of *G'* and *G"* versus frequency for A grade MCC with varying concentrations exhibited the similar pattern. Other grades AaltoCell™ MCC tend the same pattern with A grade MCC and the results are presented in Appendices. The magnitude of *G'* and *G"* were proportional to the concentrations and, the other grades of AaltoCell™ MCC had the similar trend as well. Furthermore, it could be observed that at higher concentration (10 % and 12 %), the values of *G"* became higher than then values of *G'*. And at 5 % concentration, it can be clearly observed that until 4.8 Hz, the values of *G'* were

higher than values of *G"* and after that, the values of *G"* were higher than values of *G'*. The values of *G'* and *G"* at 2.4 % concentration were quite similar with reported in Rudraraju's paper (Rudraraju, Wyandt 2005b) for 2 % (w/w) MCC/NaCMC. At this low concentration, the entanglement of microstructure is not strong enough to against large shear forces.

4.4. Viscosity Test

The effect of different grades and varying concentrations on viscosity are presented in Figure 11. The viscosity is the resistance of a fluid to flow and in the shear deformation viscosity is the ratio of applied shear stress to resulting shear rate. The changes of the viscosity with shear rate are related to the orientation or deformation of molecule network in the direction of flow (GHANNAM, ESMAIL 1997).

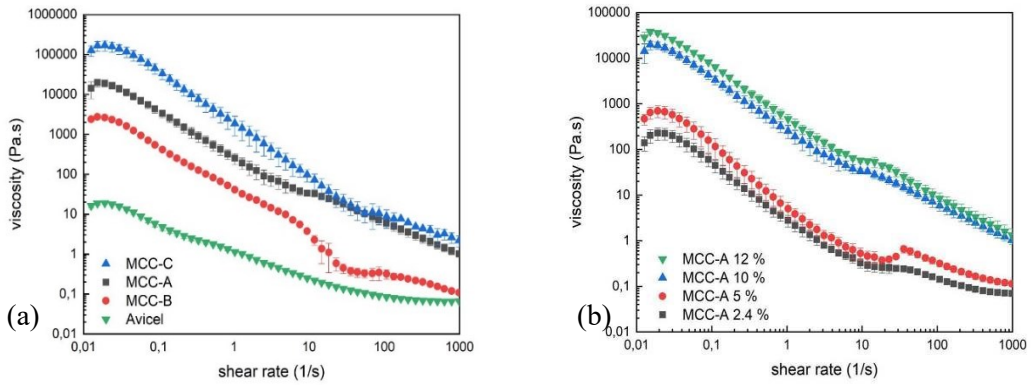

Figure 11. Influence of shear rate on the viscosity of MCC hydrogels, a, at varying grades; b, at varying concentrations.

According to the Figure 11a, all grades of MCC can be observed the shear thinning behavior, which means the values of viscosity decrease with the increase of shear rate. The C grade MCC exhibits the highest viscosity which is decade higher than the A grade MCC. Avicel® has the lowest viscosity, which means that Avicel® is the most liquid-like sample. The effect of varying concentrations of grade A MCC on the viscosity can be observed from Figure 11b. The viscosity is strongly dependent on the concentration. With the increase of concentration, the value of viscosity correspondingly increases. Other grades AaltoCell™ MCC tend the same pattern with A grade MCC and the results are presented in Appendices. Compared with 2 % (w/w) MCC/NaCMC hydrogels reported in the literature (Rudraraju, Wyandt 2005a), the values of viscosity were quite similar, whereas the

MCC/NaCMC hydrogels also exhibit the shear thinning behavior. The shear thinning behavior happens during the increase of shear rates. At very low shear rates, the material flow is classified as the first Newtonian plateau where the microstructure is random, and viscosity is constant due to the Brownian diffusion. When shear rate increases, the shear force imposes order, particles align along the flow direction and viscosity decreases. Finally, the maximum order is achiever, and the viscosity becomes constant again.

4.5. Drug Release Experiments

The drug release experiments were performed to study the effect of different grades of MCC on the release profiles of two different model compounds from hydrogels. From Figure 12, it can be observed that AaltoCell™ MCC hydrogel matrix did not swell in the PBS buffer during the release experiments. Therefore, it could be assumed that the diffusion in the hydrogel matrix would control the rate of drug release which can be beneficially utilized in controlled drug delivery applications.

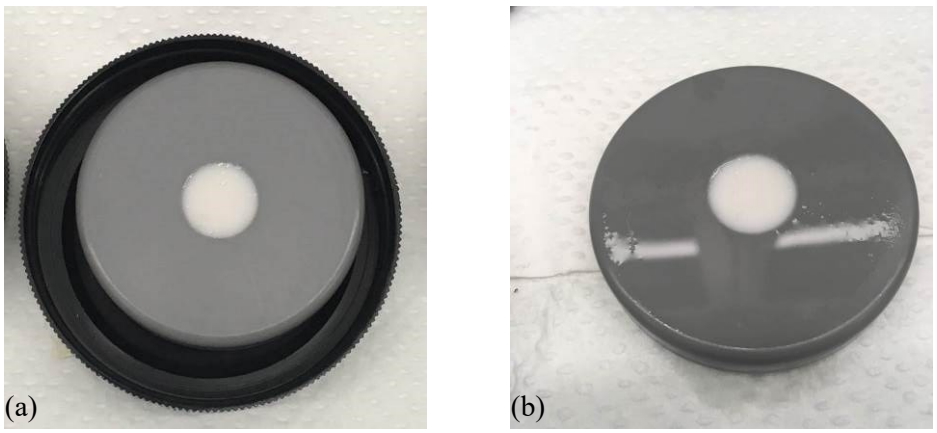

Figure 12. Comparison of disc mold before and after experiment. a, the disc mold before experiment; b, the disc mold after experiment.

The results of the drug release experiments are presented in Figure 13. The drug release profiles for both metronidazole and lysozyme were quite similar from the A grade and B grade MCC hydrogels. Relatively similar drug release profile for metronidazole was obtained from C grade MCC hydrogel. However, the release profile of lysozyme from C grade MCC hydrogel was significantly different from other grades MCC hydrogel. According to the figure, A grade MCC had the fastest drug release followed by B grade MCC and C grade MCC had the lowest drug release for both metronidazole and lysozyme. The release of metronidazole reached 100 % at 48 h from A grade MCC and reached 100 % from B grade MCC at 144 h.

However, the release from C grade MCC only reached 95 % at the end of experiment. The release of lysozyme was much lower and less than the metronidazole, which was caused by the different molecular weight and sizes of molecule. The release of lysozyme reached 80 % and 72.4 % from A grade and B grade MCC hydrogels at the end of experiment, however, lysozyme only released 50 % from C grade MCC. Compared with literature (Paukkonen, Kunnari et al. 2017), the release of metronidazole from pure ANFC hydrogels was relatively similar, however, the release of lysozyme from ANFC hydrogels was lower than the release from all grades AaltoCell™ MCC. The cationic charge of lysozyme reduced the rate of release by interaction with ANFC. Furthermore, the low release of lysozyme from C grade MCC was caused by the lignin in the formulation, which can react with protein, thus, reduced the amount of release.

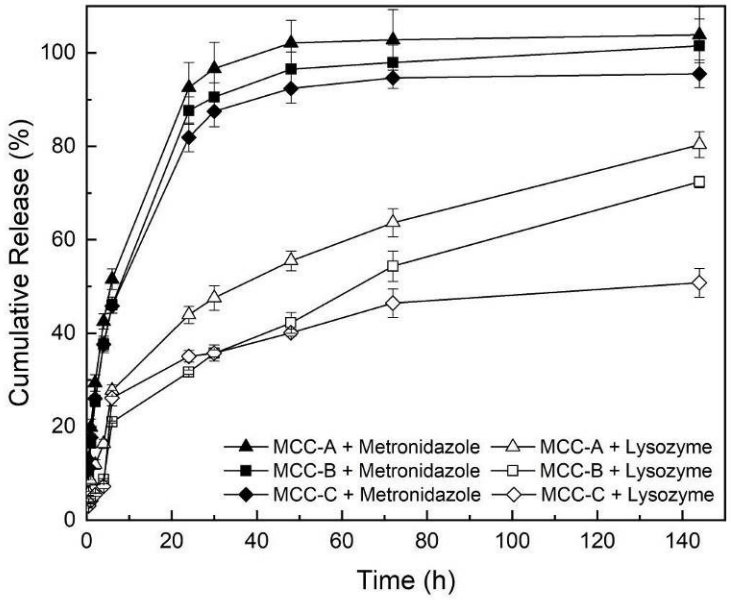

Figure 13. Cumulative release curves of varying formulations.

The Fickian model was used to calculate diffusion coefficients inside the hydrogels for both metronidazole and lysozyme Equation 2. It was considered that the initial concentration of metronidazole was not over the solubility of metronidazole, thus, the Fickian model was suitable to calculate the diffusion coefficient for metronidazole. Significantly different diffusion coefficients for metronidazole and lysozyme can be observed from Table 3 the order of magnitude for diffusion

coefficients in different grades MCC was A grade MCC > B grade MCC > C grade MCC for both metronidazole and lysozyme. The diffusion coefficients for lysozyme in A, B and C grade MCC were 38, 24 and 21 × 10^{-8} cm^2/s respectively, which were decade lower than diffusion coefficients for metronidazole. Compared with literatures (Koutsopoulosa, Unsworth et al. 2009, Paukkonen, Kunnari et al. 2017), the diffusion coefficient of lysozyme is around 100 × 10^{-8} cm^2/s in water and below 5 × 10^{-8} cm^2/s in ANFC hydrogels. In addition, diffusion coefficients of metronidazole in all grades of AaltoCell™ MCC were lower than the reported in ANFC hydrogels as well. These differences indicated that AaltoCell™ MCC hydrogels provided a significant control for the diffusion of both lysozyme and metronidazole. Diffusion coefficients are also affected by the properties of drugs. Metronidazole shows neutral charge at pH 7.4, whereas lysozyme is positive charge, may bind to anionic matrices by electronic forces. This could be the reason of the low diffusion coefficient of lysozyme in ANFC hydrogels. Also different grades of AaltoCell™ MCC matrices exhibit different viscosities, which are reflected on the release profiles. The viscosity result of different formulations is shown in Appendices. The MCC-C formulations presented highest viscosity, which also had the lowest release rates of both metronidazole and lysozyme from this matrix.

Table 3. Diffusion coefficients for model compounds in varying grades of AaltoCell™ MCC

Compound	**Diffusion coefficients for model compounds in varying grades of AaltoCell™ MCC (10^{-8} cm^2/s)**		
	MCC – A	**MCC – B**	**MCC – C**
Metronidazole	269	237	215
Lysozyme	38	24	21

5. Conclusion and Recommendations

Three grades of AaltoCell™ microcrystalline cellulose were characterized and applied for drug delivery studies. The characterizations were focused on the rheological properties and compared with commercial microcrystalline cellulose, Avicel® PH-101. The oscillatory stress sweep, frequency sweep and viscosity test were carried out on the different concentrations of AaltoCell™ MCC and varying grades MCC. The results indicate that the rheological properties strongly depend on the concentration of AaltoCell™ MCC, which means with the increase of concentration, the rheological properties are significantly improved. Moreover, compared with Avicel®, the AaltoCell™ MCC was stable at hydrogel at room temperature with promising rheological properties without any chemical modification.

According to the result of drug release experiment, the AaltoCell™ MCC was indicated that did not swell in the PBS buffer, which can be beneficially utilized in the controlled drug delivery. In addition, the diffusion coefficients of metronidazole and lysozyme from AaltoCell™ MCC hydrogels indicated that the AaltoCell™ MCC hydrogels efficiently controlled the diffusion of both large and small molecule which shows great potential in the drug delivery application.

The AaltoCell™ MCC tends promising potential in industrial applications; many researches can be carried out. For instance, the present study of AaltoCell™ MCC is only focused on two drug compounds, the release profiles of more model compounds can be studied in the future studies. In addition, the effect of AaltoCell™ MCC hydrogel concentration on the release profiles can be tested. It can be foreseen that if AaltoCell™ MCC can be used in industrial manufacturing, the production costs can be reduced due to the simplified process.

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Appendices

Appendix 1. Rheology data of AaltoCell™ MCC and its Formulations

Figure 14 – 19 present rheological results of grade B and C AaltoCell™ MCC with different concentrations. Figure 14 and Figure 17 are results of oscillatory stress sweep, whereas Figure 15 and Figure 18 show the results of frequency sweep, and Figure 16 and Figure 19 present viscosities of B and C grades AaltoCell™ MCC. Figure 20 presents viscosity result of 12 % AaltoCell™ MCC hydrogel formulations.

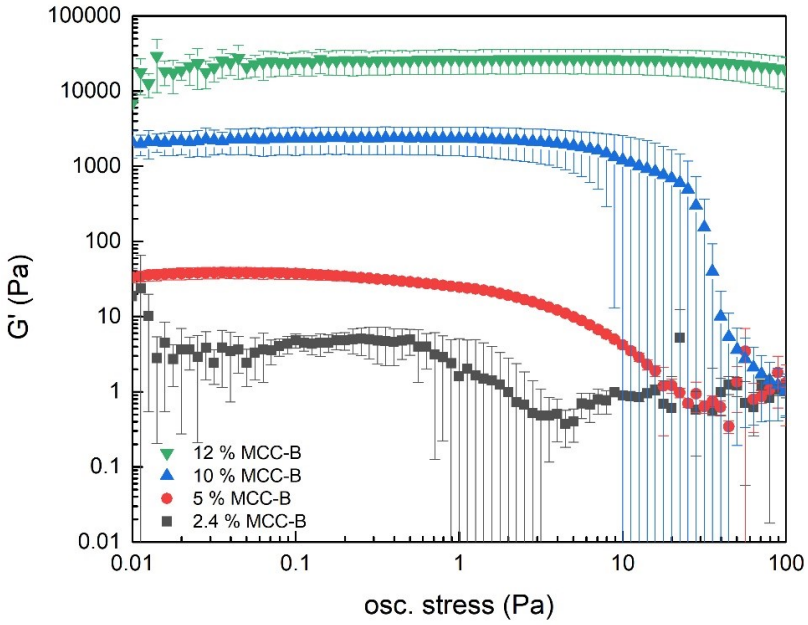

Figure 14. Effect of oscillation stress on the elastic modulus (*G'*) of B grade MCC at varying concentrations.

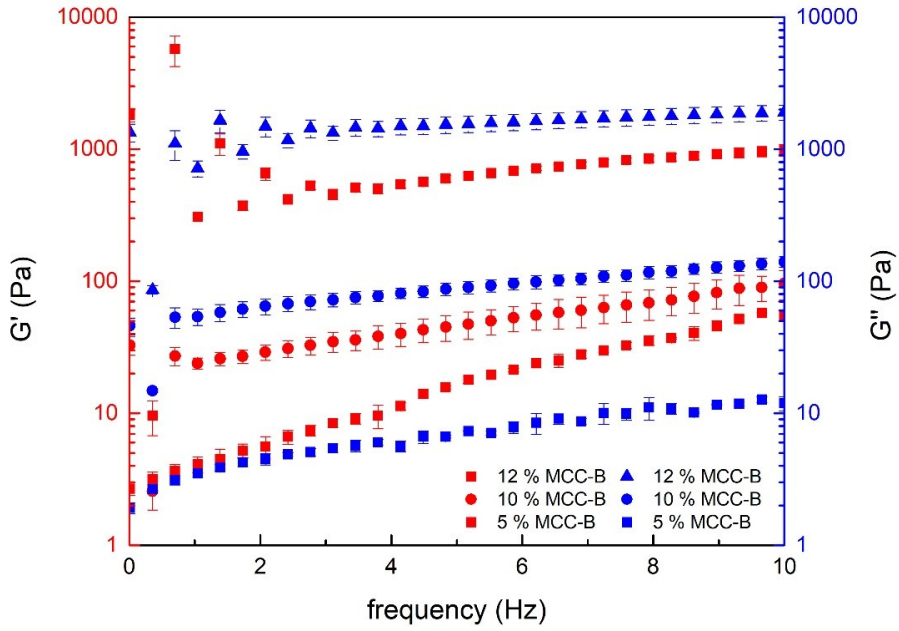

Figure 15. Influence of frequency on the storage modulus (*G'*) and loss modulus (*G''*) of B grade MCC at varying concentrations.

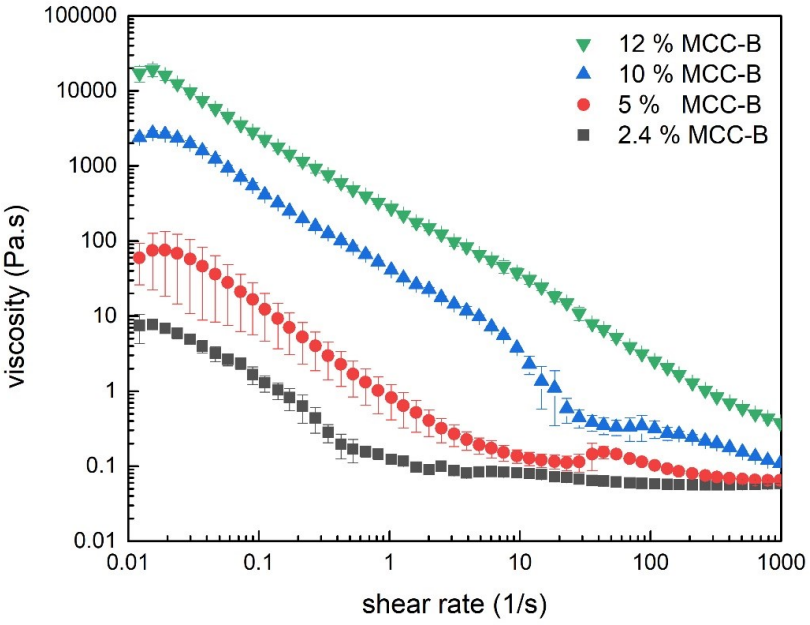

Figure 16. Influence of shear rate on the viscosity of B grade MCC at varying concentrations.

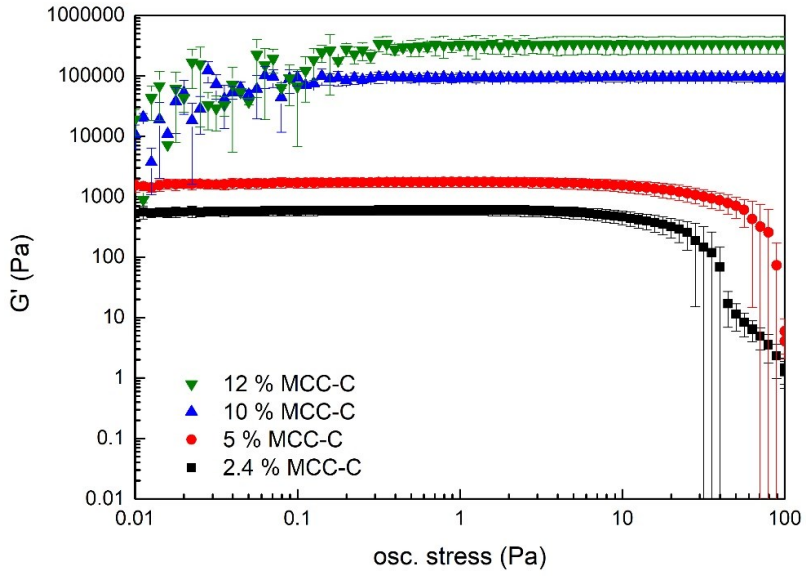

Figure 17. Effect of oscillation stress on the elastic modulus (*G'*) of C grade MCC at varying concentrations.

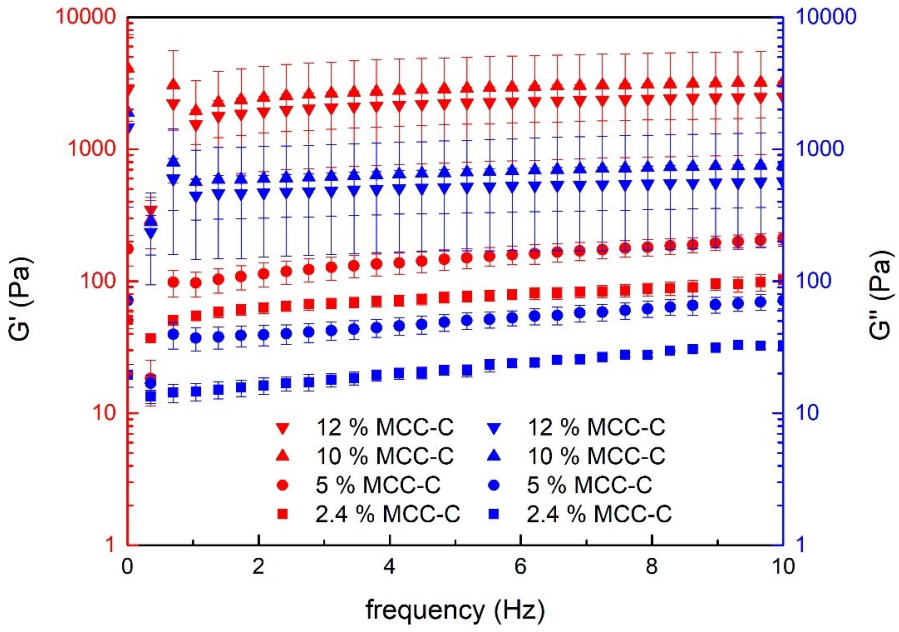

Figure 18. Influence of frequency on the storage modulus (*G'*) and loss modulus (*G''*) of C grade MCC at varying concentrations.

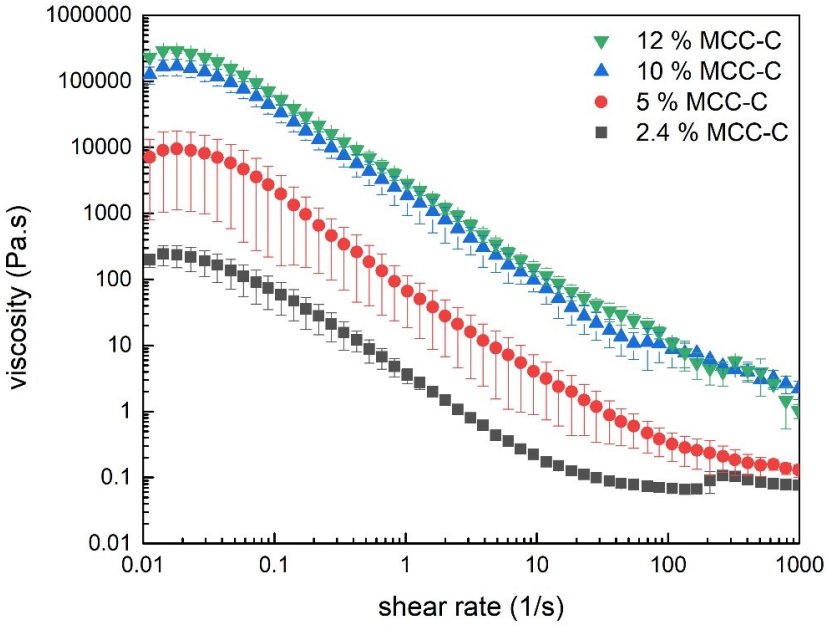

Figure 19. Influence of shear rate on the viscosity of B grade MCC at varying concentrations.

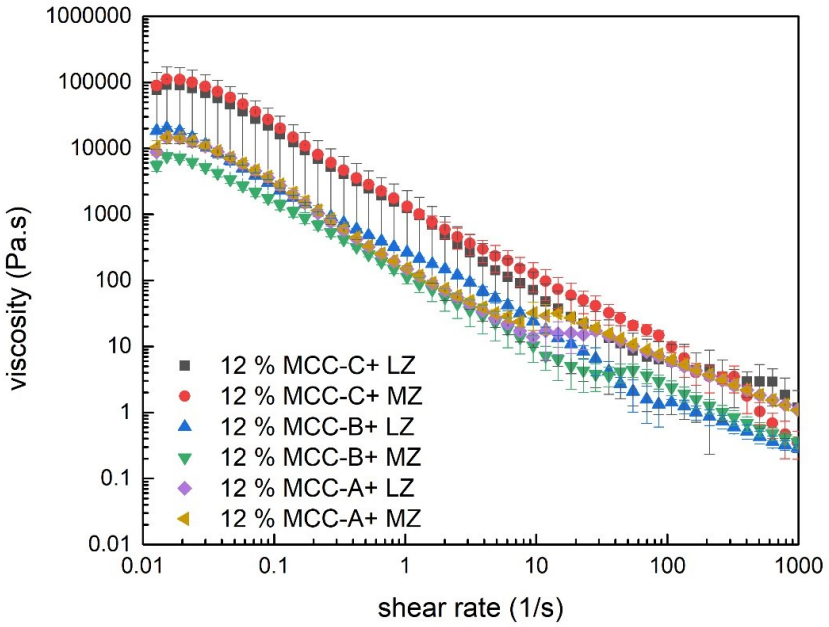

Figure 20. Influence of shear rate on the viscosity of 12 % AaltoCell™ MCC hydrogels at varying formulations.

Appendix 2. Calibrations of Absorbance Measurement

The calibration was carried out with stock solutions for metronidazole and lysozyme. For metronidazole, absorbance was measured at 320 nm from 3 μg/ml to 35 μg/ml. For lysozyme, absorbance measurement was carried out at 280 nm from 2.5 μg/ml to 30 μg/ml. Calibrations were done twice for each drug, and the results listed in Table 4 and Table 5 were the average of twice measurements.

Table 4. Calibration data of metronidazole absorbance measurement.

Sample	μg/ml	abs
Std 1	3	0.1549
Std 2	5	0.2573
Std 3	8	0.4157
Std 4	10	0.5204
Std 5	15	0.7889
Std 6	20	1.0550
Std 7	25	1.3045
Std 8	30	1.5581
Std 9	35	1.8076

Table 5. Calibration data of lysozyme absorbance measurement.

Sample	μg/ml	abs
Std 1	2.5	-0.0006
Std 2	5	0.0023
Std 3	8	0.0087
Std 4	10	0.0129
Std 5	15	0.0233
Std 6	17	0.0284
Std 7	20	0.0347
Std 8	25	0.0449
Std 9	30	0.0580

According to the data in Table 4 and Table 5, standard curves for absorbance measurements of metronidazole and lysozyme can be established.